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USE OF VEGETATED AGRICULTURAL DRAINAGE DITCHES TO DECREASE TOXICITY OF IRRIGATION RUNOFF FROM TOMATO AND ALFALFA FIELDS IN CALIFORNIA, USA

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Abstract—The current study investigated the potential of vegetated drainage ditches for mitigating the impact of agricultural irrigation runoff on downstream aquatic ecosystems. Water column toxicity to larval fathead minnow (*Pimephales promelas*),and the amphipod *Hyalella azteca* was measured for 12 h or less at the ditch inflow and outflow, using custom-built in situ exposure systems. In addition, water and sediment samples were subject to standard toxicity tests with *Ceriodaphnia dubia* and *H. azteca,* respectively. No acute toxicity to larval fathead minnow was observed; however, runoff was highly toxic to invertebrates. Passage through a 389- to 402-m section of vegetated ditch had a mitigating effect and reduced toxicity to some degree. However, runoff from an alfalfa field treated with chlorpyrifos remained highly toxic to both invertebrate species, and runoff from a tomato field treated with permethrin remained highly toxic to *H. azteca* after passage through the ditch. Predicted toxic units calculated from insecticide concentrations in runoff and 96-h median lethal concentration (LC50) values generally agreed with *C. dubia* toxicity measured in the laboratory but significantly underestimated in situ toxicity to *H. azteca*. Sediments collected near the ditch outflow were toxic to *H. azteca.* Results from the current study demonstrate that experimental vegetated ditches were unable to eliminate the risk of irrigation runoff to aquatic ecosystems. In addition, protective measures based on chemical concentrations or laboratory toxicity tests with *C. dubia* do not ensure adequate protection of aquatic ecosystems from pyrethroid-associated toxicity. Environ. Toxicol. Chem. 2010;29:2859–2868. © 2010 SETAC

Keywords—Permethrin Chlorpyrifos Agricultural runoff *Hyalella azteca* *Ceriodaphnia dubia*

INTRODUCTION

Offsite movement of insecticides, in particular organophosphates (OPs) and pyrethroids, in storm water and irrigation runoff has contributed to the contamination and toxicity of surface waters in agricultural areas around the world [1–3]. All OPs and pyrethroids are potent neurotoxicants [4,5], and aquatic organisms, especially insects, crustaceans, and fish, are highly sensitive to these chemicals. Acute toxicity to aquatic invertebrates is observed at concentrations below 1 μg/L [1,6]. Whereas fish are generally less sensitive to OPs ([7]; http://cfpub.epa.gov/ecotox, accessed 2009), they are highly sensitive to pyrethroids [6]. Sublethal toxic effects measured in fish include impairment of sensory nerves and corresponding changes in behavior [8], abnormal swimming [9], immunosuppressive effects [10], and endocrine disruption [11,12].

The widespread application of OP and pyrethroid insecticides in California's Central Valley (USA), a region dominated by agriculture, has resulted in the placement of numerous water bodies on the State's Clean Water Act 303(d) list because of pesticide impairment. In 1999, the California State Water Resources Control Board instituted a mandate to reduce pesticide-contaminated runoff entering surface waters ([13]; http://www.swrcb.ca.gov/water_issues/programs/bptcp/index.shtml). Although the use of OPs, in particular diazinon and chlorpyrifos, has since declined, the application of pyrethroid insecticides has become increasingly prevalent [14]. In 2005, five pyrethroids were among the top 21 agricultural insecticides by acres treated in California: lambda-cyhalothrin, permethrin, esfenvalerate, cypermethrin, and cyfluthrin (California Department of Pesticide Regulation, Pesticide Use Reporting database, www.cdpr.ca.gov). Based on their chemical characteristics, pyrethroid insecticides are considered less mobile and less persistent in the environment than OPs.

Best management practices therefore have been advocated to reduce contamination and prevent impairment of aquatic ecosystems caused by insecticides. In particular, passage of agricultural runoff through vegetated agricultural drainage ditches or constructed wetlands has been shown to effectively reduce pesticide load in irrigation runoff [15–18]. Ditch vegetation, in particular, proved to be a major sink for several OPs and pyrethroids. However, invertebrate toxicity remained high in experiments in which pesticide load was significantly reduced [19], demonstrating the need for toxicity tests when evaluating the mitigating effects of best management practices.

The goal of the current study was to validate the use of vegetated agricultural drainage ditches as a best management practice for mitigation of aqueous residues of selected OP and pyrethroid insecticides of regulatory concern. We used in situ or laboratory tests with larval fathead minnow (*Pimephales promelas*), the amphipod *Hyalella azteca*, and the waterflea

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Ceriodaphnia dubia to compare toxicity of irrigation runoff between inflow and outflow of 389- to 402-m-long ditch segments. In addition, we measured the toxicity of ditch sediments near the outflow before and after runoff occurred. Besides being commonly used in standard toxicity tests across the United States, the test species are resident in surface waters throughout California.

MATERIALS AND METHODS

Experimental sites

Study sites were two ditches receiving irrigation runoff from two agricultural fields in Yolo County, California; a 7-ha alfalfa field (site 1) and a 6-ha tomato field (site 2). Each field had existing drainage ditches in place. Length, width, and depth of experimental ditches were 402, 1.5, and 0.6 m (site 1), and 389, 4.9, and 1.5 m (site 2). These were planted with plugs of creeping wild rye (*Leymus triticoides*) and slender sedge (*Carex praegracillis*). One day before the irrigation events, plant density and percent plant cover were recorded in three replicate quadrants (0.25 m^2) and showed approximately 100% and 50% cover at sites 1 and 2, respectively (Supplemental Data, Table S1).

The soil type at site 1 was Myers clay (fine smectitic, thermic aridic haploxererts). Soil analyses conducted by the University of California Agriculture and Natural Resources Analytical Laboratory indicated a soil pH of 7; particle size distribution of 21% sand, 35% silt, and 44% clay; cation exchange capacity of 35.5 mEq/100 g; 0.6% organic carbon; and 27.6% soil moisture. At site 2, soil consisted of a mixture of Sycamore silt–clay loam (fine-silty, mixed, superactive, nonacid, thermic mollic endoaquepts) and Brentwood silt–clay loam (fine, smectitic, thermic typic haploxerepts). Soil analyses indicated a pH of 7.6; particle size distribution of 13% sand, 52% silt, and 35% clay; cation exchange capacity of 31.2 mEq/100 g; 0.47% organic carbon; and 32.8% soil moisture.

Approximately 12 h before irrigation, the alfalfa field (site 1) was treated with chlorpyrifos (Lock-On™, Dow AgroSciences) at the manufacturer-recommended rate of 42 mg/m^2 active ingredient. The tomato field (site 2) was treated with permethrin (Perm-UP 3.2 EC™, United Phosphorus) applied at a rate of 22 mg/m^2 active ingredient.

Test organisms

Hyalella azteca were obtained from Aquatic Research Organisms. Adult amphipods were used for in situ exposures, because smaller animals were able to escape from flow-through cages. These were acclimated in nonchlorinated well water from the Center for Aquatic Biology and Aquaculture at University of California-Davis (UCD-ATL control water, electrical conductivity [EC] 677 μS/cm, T = 25°C) for a minimum of 5 d before each experiment. Sediment tests followed standard protocols [20]. Amphipods (7–14 d old) were acclimated in reconstituted water, amended with dry salts to attain U.S. Environmental Protection Agency (U.S. EPA) moderately hard specifications (hardness 90–100 mg/L $CaCO_3$, alkalinity 50–70 mg/L as $CaCO_3$, EC 330–360 μS/cm and pH 7.8–8.2) [20,21]. During acclimation, amphipods were fed Tetramin flakes (Tetra®) daily. Adult *H. azteca* that passed through a 750-μm mesh but were retained by a 560-μm mesh were used for in situ experiments. Larval *P. promelas* were obtained from Aquatox. For in situ exposures, 10-d-old larvae were acclimated to UCD-ATL control water (T = 25°C) for at least 24 h. For 96-h tests performed at AquaScience (Davis, CA, USA), *P. promelas* were acclimated to laboratory conditions for at least 24 h in reverse osmosis and granular carbon-treated well water adjusted to U.S. EPA moderately hard specifications [21]. Laboratory tests were initiated with 6- to 8-d-old fish. During acclimation, fish were fed *Artemia* nauplii twice daily and 1 h before initiation of the test. *Ceriodaphnia dubia* were collected from cultures housed at AquaScience.

Sample collection for analytical chemistry and laboratory tests

Water grab samples for 96-h laboratory tests with *C. dubia* and *P. promelas* and analytical chemistry were collected on July 19, 2007 (site 1), and August 16, 2007 (site 2), at the beginning of flow, during peak flow, and at the end of the runoff events. Water samples for analytical chemistry were collected at 2-h intervals. Subsurface samples were collected using amber prelabeled, acid-washed glass bottles (3.8 L). Sediment samples were collected from experimental sites near the outflow of the vegetated ditch 48 h or less before irrigation runoff occurred (*pre-irrigation*). Postirrigation samples were collected in the same reach within 24 h of the cessation of runoff. Additional postirrigation samples were collected 14 (site 1) or 7 (site 2) days later. Sediment (~8 L per sample) was collected from the top 5 cm using precleaned stainless steel spoons ([22]; http://www.waterboards.ca.gov/water_issues/programs/swamp/docs/qapp/qaprp082209.pdf) and acid-washed 8-L plastic buckets. Samples were transported on wet ice to the UC Davis Aquatic Toxicology Laboratory (UCD-ATL), and stored in the dark at 4°C (water) or frozen (sediment). For analytical chemistry samples were shipped on ice to the Water Pollution Control Laboratory, California Department of Fish and Game (Rancho Cordova, CA, USA) within 2 d of collection.

Toxicity testing with larval fathead minnow and H. azteca

Exposure system. Custom-built exposure chambers were used for toxicity monitoring. For each experiment, three in situ systems were set up: at the ditch inflow, at the ditch outflow, and a control system close to the inflow location. The inflow and outflow systems were located 1 m upstream and downstream of the vegetated areas, respectively.

Each system consisted of a pump assembly, a delivery system, an exposure chamber, and a drainage system (Fig. 1). Before use, all system parts were leached in tap water for a minimum of 96 h, and fresh tap water was pumped daily through each device for a week before use. Water was pumped into the exposure systems through polyvinyl chloride hoses (~7 m) as soon as water began flowing in the ditch. Collection containers were installed approximately one week before the experiments. Low-density polyethylene liners, which were renewed immediately before runoff occurred, prevented contamination. Flow was maintained at 250 to 300 L/h. Water was delivered to the bottom of the chamber and exited at the top to prevent stratification. The chamber consisted of a high-density polyethylene rectangular tank divided into six sections. Exposure cages for *H. azteca* (polypropylene jars and 425 μm mesh) and *P. promelas* (low-density polyethylene cap and 425-μm mesh) were custom made. All cages were leached in UCD-ATL control water for a minimum of 96 h. Sets of caged test organisms (*P. promelas, H. azteca*) were deployed every 2 h into one section and monitored for survival at 2-h intervals. The chamber was covered with a clear acrylic sheet.

Control and ambient systems were identical, except that control water was recirculated through a high-density polyethylene rectangular sump with lid. To mimic conditions in ambient systems, the sump contained a 20-L bucket with liner

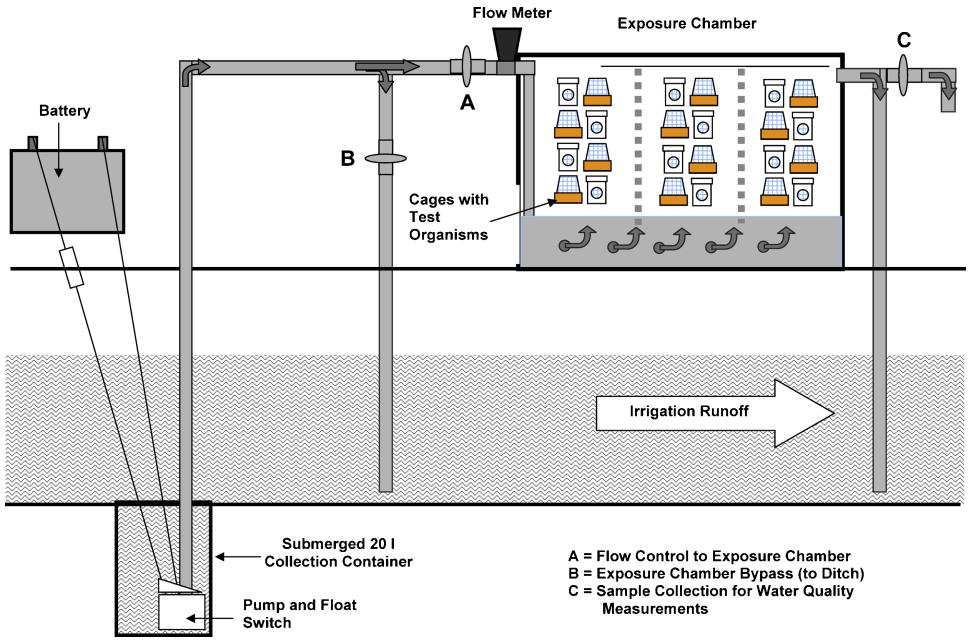

Fig. 1. In situ system used for flow-through exposures of larval *Pimephales promelas* and *Hyalella azteca* to agricultural irrigation runoff. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com.]

and the complete pump assembly. Tubing length was the same in all exposure systems.

In situ exposure. Less than 8 h before exposure experiments, organisms were randomly distributed to four replicate exposure cages containing five animals each, at the UCD-ATL, and transported to the field sites in control water maintained at $\leq$25°C. The first set of test organisms was deployed 15 min after the pumps started and the exposure system had filled. Additional sets of four replicate cages and organisms were deployed every 2 h until runoff ceased at the inflow (8 h, four deployment groups; site 2), or a maximum of 10 h (five deployment groups; site 1). Survival of exposed organisms was recorded every 2 h. At termination of in situ exposures, surviving organisms were moved to control water at $\leq$25°C and transported to UCD-ATL. This meant that organisms deployed last were exposed to runoff for only 2 h. Water was renewed on arrival at the laboratory, and temperature maintained at 25°C for the remainder of a 48-h period.

At site 1, all systems were started simultaneously, and exposures began when runoff water first reached the ditch outflow. At site 2, systems started automatically when water arrived at the respective location (inflow, outflow) of the ditch, which occurred 2 h apart. The control system was started simultaneously with the inflow system.

Water quality and flow measurements

Temperature and water flow in exposure systems were measured continuously, and dissolved oxygen (DO), specific conductivity, EC, and pH were recorded at 2-h intervals using YSI 85 meters, and a Beckman 240 pH meter. Meters were calibrated according to the manufacturer's instructions on each sampling day. Battery-operated aquarium air pumps were used to aerate the water when DO fell below 4 mg/L. Data are provided in the Supplemental Data, Table S2. Ambient water temperature and EC ranges were 17 to 30°C and 288 to 495 μS/cm, respectively, at site 1, and 26 to 31°C and 323 to 468 μS/cm, respectively, at site 2. The pH range at both sites was 7.4 to 8.4, and DO ranges were 2.5 to 5.5 (site 1) and 5.0 to 8.1 (site 2). Water temperature in the control system was adjusted to temperature in ambient systems using an ice or warm water bath. All other parameters in control systems were within the acceptable range of *H. azteca* [20].

Laboratory tests with P. promelas *and* Ceriodaphnia dubia

Acute 96-h toxicity tests were initiated within 24 h of sample collection and followed standard U.S. EPA protocols [21]. Moderately hard treated well water [20,21] was used as control and dilution water. Test treatments were: 0 (control), 1, 5, 10, 25, 50, and 100% of runoff sample. Test solutions were renewed and mortality recorded daily. *Ceriodaphnia dubia* tests were initiated with younger than 24-h-old neonates. Each water sample was tested using four 20-ml glass vials each containing 18 ml test solution and five neonates. *C. dubia* were fed a mixture of green algae (*Pseudokirschneriella subcapitata*) and a mixture of yeast, organic alfalfa, and trout chow 4 h before water renewal. Larval *P. promelas* tests consisted of four replicate 400-ml glass beakers each containing 250 ml test solution and 10 fish (6–8 d old). Fish were fed *Artemia* nauplii daily, 4 h before water renewal. Acute *P. promelas* and *C. dubia* tests were conducted at 25 ± 2°C with a 16:8 h light:dark photoperiod. The test was acceptable if control survival was at least 90%.

Temperature, DO, pH, alkalinity, hardness, conductivity, and ammonia of water samples were measured on arrival at the laboratory. Meter calibration and water quality measurements followed manufacturer-recommended procedures. Water temperature was continuously recorded using a circular chart recorder (Dickson, Model ICT855). In addition, DO (YSI Model 550A), pH (Beckman 240), temperature, and conductivity (WTW Model 330) were measured in the initial and 24-h renewal test solutions. Alkalinity (Hach Model AL-DT) and hardness (Hach HA-DT) were measured using Hach colorimetric tests (Hach). Total ammonium was measured using a Hach DR-700 colorimeter.

Laboratory sediment tests with H. azteca

Ten-day sediment toxicity tests were initiated within 14 d of sample collection and followed standard U.S. EPA protocols [20]. Before testing, samples were homogenized and sieved

(1-mm mesh) to remove debris and larger organisms, then 100 ml sediment and 175 ml overlying water was added to eight 300-ml beakers. Moderately hard reconstituted laboratory control water [20] was used as overlying water. At test initiation, 10 randomly selected 7- to 14-d-old *H. azteca* were placed into each beaker. An additional 80 organisms were preserved for initial weight measurements. Tests were performed at $23 \pm 2^{\circ}C$ with a 16:8 light:dark photoperiod. The overlying water was renewed twice daily approximately 8 h apart. Organisms were fed daily with 1.0 ml of a mixture of yeast, organic alfalfa, and trout chow [20] after the second water renewal. Mortality and amphipod behavior (e.g., sediment avoidance) were recorded daily. At test termination (day 10), surviving amphipods were counted, dried at 80°C for at least 12 h then weighed on a Mettler AE-163 balance to the nearest 0.01 mg. Hardness, alkalinity, conductivity, pH, and ammonia were measured at test initiation and termination, and DO and temperature were measured daily using equipment described previously.

Control sediment used in these tests was collected in June 2007, following methods described from three fallow agricultural fields on the UC Davis campus. These sites were selected based on the comparability of their soil types with that of the study sites and were identified on soil maps for Yolo County (National Resource Conservation Service). Site 1 soil type was identified as Myers clay, whereas site 2 soil type was identified as a combination of Sycamore silty clay loam and Brentwood silty clay loam. The soil used for reference sediment was a combination of Brentwood silty clay loam and Yolo silt loam. Before use in toxicity tests, reference sediment was homogenized, sieved, and subject to a 10-d *H. azteca* toxicity test to ensure that it was nontoxic.

Control experiments

Two experiments were conducted to address concerns regarding the adequate interpretation of results with respect to the temperature fluctuations experienced by the exposed test organisms during in situ exposures, and adsorption of pesticides to in situ exposure systems.

Temperature tolerance of H. azteca*.* To determine whether high water temperatures or extreme temperature fluctuations could affect *H. azteca* survival, organisms were exposed to 25°C, 32°C, or fluctuating temperatures for 48 h. Four replicate in situ cages, each containing five organisms, were placed into 4 L of control water maintained at 25°C or 32°C. In temperature fluctuation tests, test cages were moved every h from 32°C to 17°C or vice versa for 10 h (approximate maximum field exposure time), then transferred to 25°C for 38 h. Measured water quality parameters were: EC 332–389 μS/cm, pH 8.0 to 8.09, and 7.4 to 8.6 mg/L DO. Organisms were fed once before the test, and survival was recorded every 2 h.

Pesticide adsorption to in situ exposure system. To quantify the amount of pesticide adsorption during passage through the exposure system, a mixture of pyrethroid insecticides and the OP insecticide chlorpyrifos were spiked into 208 L control water at a nominal concentration of 400 ng/L each. Water was pumped through at 200 L/h. Samples were collected before (baseline) and after passage through the system when water first drained from the exposure chamber (time 0), and 12 and 24 min thereafter. Before liquid–liquid extraction, each water sample was spiked with isotopically labeled permethrin to account for differences in extraction efficiency. Concentrations were calculated as a percentage of baseline concentrations.

Analytical chemistry

Water extraction. Water sample extraction for gas chromatography analysis by U.S. EPA Method 8081B and 8141B followed U.S. EPA Method 3510C separatory funnel liquid–liquid extraction. Water samples were fortified with surrogates (triphenyl phosphate and dibromooctafluorobiphenyl) and extracted twice with dichloromethane. Extracts were dried using sodium sulfate, concentrated, and solvent exchanged with petroleum ether. Water sample extraction for analysis by liquid chromatography-tandem mass spectrometry followed U.S. EPA Method 3535A solid- phase extraction. Water samples were fortified with appropriate isotope-labeled surrogates and extracted using solid-phase extraction with Waters HLB® solid-phase extraction cartridges.

Sediment extraction. Sediment sample extraction followed U.S. EPA Method 3545A pressurized fluid extraction. Homogenized sediment (10 g) samples were mixed with pre-extracted Hydromatrix® (7 g, Varian) and fortified with surrogates (triphenyl phosphate, dibromooctafluorobiphenyl, and dibutyl chlorendate). Samples were extracted twice with acetone/dichloromethane (50/50, v/v) using a Dionex accelerated solvent extractor (ASE 200, 100°C, 1500 psi). Extracts were dried using sodium sulfate, concentrated and solvent exchanged with petroleum ether. Cleanup of sulfur and other matrix interferences followed U.S. EPA Method 3600C.

Analysis. Water and sediment extracts were analyzed for chlorpyrifos, methyl parathion, and diazinon using U.S. EPA Method 8141B, and for permethrin, bifenthrin, lambda cyhalothrin, dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), and dieldrin using U.S. EPA Method 8081B. Organophosphates were analyzed by dual-column high-resolution gas chromatography with flame photometric detectors in phosphorous mode. Pyrethroids and organochlorines were analyzed using dual-column high-resolution gas chromatography equipped with electron capture detectors. Atrazine, trifluralin, fipronil, fipronil sulfone, alachlor, metolachlor, cyanazine, and pendimethalin were analyzed using high-performance liquid chromatography–tandem mass spectrometry.

Data analysis

Analyses were performed using JMP 5.0.1, CETIS version 1.7 (Tidepool Scientific) and ToxCalc (Tidepool Scientific). *Hyalella azteca* survival data from in situ experiments were analyzed for each 2-h time point, using a one-way analysis of variance with Tukey's multiple comparison procedure (two-tailed $p = 0.05$). For each deployment group, linear interpolation methods of the time series data (survival after 2 h, 4 h, and so forth) were analyzed to determine *effective exposure time*, which is defined as the exposure time that resulted in 50% amphipod mortality (ET50). In cases in which less than 50% mortality occurred during in situ exposure to runoff, the exposure time that resulted in 50% amphipod mortality was expressed as *time of exposure to runoff*, as well as calculated using 24- and 48-h survival recorded after transfer to control water. The *t* test was used to determine differences in toxicity between inflow and outflow locations. Data from laboratory tests were analyzed using U.S EPA standard statistical protocols [20,21]. For *C. dubia* data, the effective exposure concentration (96-h LC50) was determined as a percentage of ambient sample. The 96-h LC50 is the calculated concentration that caused 50% mortality of the test organisms within 96 h.

Measured toxic units (TU) for *C. dubia* were calculated from the 96-h LC50 value as follows: Measured TU = 100/96-h LC50 (% ambient sample); a 96-h LC50 of 100% would result in TU = 1. For *H. azteca* survival in situ, measured TUs were calculated as follows: TU in situ = 96/LT50 (h), where an LT50 = 96 h would result in a TU of 1. For control animals surviving the entire 48-h experimental period, this approach resulted in less than 2 TU. Species-specific *predicted TU* were calculated as follows: Predicted TU = measured chemical concentration (μg/L)/96-h LC50 (μg/L); using the measured concentrations of chlorpyrifos or permethrin in ambient samples, and *C. dubia* 96-h LC50 for chlorpyrifos (0.08 μg/L; J. Miller, Aqua-Science, personal communication) and permethrin (0.24 μg/L) [23], or the *H. azteca* 96-h LC50 for chlorpyrifos (0.186 μg/L; I. Werner, UCD-ATL, unpublished data) and permethrin (0.078 μg/L; I. Werner, UCD-ATL, unpublished data).

Quality assurance/quality control

Toxicity tests. Quality assurance and quality control procedures for toxicity testing and analytical chemistry followed established protocols [20–22]. Reference toxicant tests were performed monthly with *P. promelas, C. dubia*, and *H. azteca,* using NaCl as the toxicant to ascertain whether organism response fell within the acceptable range as dictated by U.S. EPA. All species performed normally in reference toxicant tests conducted during the project period, July 1 to August 31, 2007. All *H. azteca* sediment tests met holding time requirements and test acceptability criteria. Average organism survival in controls was 89.6%, and average weight of control organisms was 0.085 mg/individual. Several deviations from quality assurance/quality control protocols occurred: Ammonia per micrometer was not measured at initiation (July 26, 2007) of a sediment toxicity test, and hardness, alkalinity, and ammonia per micrometer were not measured at initiation (August 22 and 30, 2007) of sediment toxicity tests.

Analytical chemistry. Nine (six duplicate and three triplicate) quality assurance/quality control samples were collected to determine the precision of analytical methods used. The average relative percent difference between duplicate samples was 19.78 ± 23.71(standard deviation [SD]) %, which is within the acceptable range of 35% or less [22]. Triplicate samples were collected and used for Matrix Spike and Matrix Spike Duplicate analyses. The average relative percent difference of these samples was 23.18 ± 19.07(SD)%, which is within the acceptable range. The acceptable range for recovery of Matric Spike/Matrix Spike Duplicate samples is 50 to 150% [22].

RESULTS

In situ toxicity testing

No significant mortality of larval *P. promelas* compared with controls was seen during in situ exposures to irrigation runoff (data not shown). In contrast, runoff was highly toxic to *H. azteca* at both experimental sites (Tables 1 and 2). Organism survival in control systems was 100% at both sites. Among amphipods exposed to irrigation runoff at site 1, 100% mortality

Table 1. Survival of *Hyalella azteca* during in situ exposure to irrigation runoff from an alfalfa field treated with chlorpyrifos (site 1; July 19, 2007)[a]

Organism deployment group	Deployment time[b] [h]	Hours after deployment [h]	Survival at inflow [%] Mean	SE	Survival at outflow [%] Mean	SE
1	6.20	0	100	0.0	100	0.0
		2	100	0.0	100	0.0
		4	100	0.0	35	9.6
		6	26	4.7	5	5.0
		8	5	5.0	0	0.0
		10[c]	0	0.0	0	0.0
		24	0	0.0	0	0.0
		48	0	0.0	0	0.0
2	8.20	0	100	0.0	100	0.0
		2	100	0.0	95	5.0
		4	10	5.8	50	19.1
		6	10	5.8	0	0.0
		8[c]	5	5.0	0	0.0
		22	0	0.0	0	0.0
		46	0	0.0	0	0.0
3	10.20	0	100	0.0	100	0.0
		2	75	9.6	95	5.0
		4	0	0.0	15	5.0
		6[c]	0	0.0	10	5.8
		20	0	0.0	0	0.0
		44	0	0.0	0	0.0
4	12.20	0	100	0.0	100	0.0
		2	48	9.5	85	9.6
		4[c]	0	0.0	5	5.0
		18	0	0.0	0	0.0
		42	0	0.0	0	0.0
5	14.20	0	100	0.0	100	0.0
		2[c]	75	9.6	75	5.0
		16	0	0.0	20	14.1
		40	0	0.0	10	10.0

[a] SE = standard error of the mean ($n = 4$).
[b] Organisms at inflow and outflow were deployed simultaneously.
[c] Remaining organisms were moved to control water and 25°C after this 2-h time interval.

Table 2. Survival of *Hyalella azteca* during in situ exposure to irrigation runoff from a tomato field treated with permethrin (site 2; August 16, 2007)[a]

Organism deployment group	Deployment time [h]		Hours after deployment	Survival at inflow [%]		Survival at outflow [%]	
	Inflow	Outflow	[h]	Mean	SE	Mean	SE
1	11:20	13:20	0	100	0.0	100	0.0
			2	100	0.0	100	0.0
			4	0	0.0	10	10.0
			6	0	0.0	0	0.0
			8[b]	0	0.0	0	0.0
			24	0	0.0	0	0.0
			48	0	0.0	0	0.0
2	13:20	15:20	0	100	0.0	100	0.0
			2	60	14.1	85	15.0
			4	5	5.0	5	5.0
			6[b]	0	0.0	0	0.0
			24	0	0.0	0	0.0
			48	0	0.0	0	0.0
3	15:20	17:20	0	100	0.0	100	0.0
			2	28	12.6	40	21.6
			4[b]	28	12.6	5	5.0
			24	10	10.0	5	5.0
			48	5	5.0	5	5.0
4	17:20	19:20	0	100	0.0	100	0.0
			2[b]	90	10.0	15	5.0
			24	66	6.9	15	5.0
			48	61	5.2	15	5.0

[a] SE = standard error of the mean ($n = 4$).
[b] After this 2-h time interval, remaining organisms were moved to control water and 25°C.

occurred within 10 h or less (Table 1), with one exception; of amphipods deployed at the outflow location 2 h before cessation of runoff, $10 \pm 20\%$ (mean $\pm$ SD) survived the 2-h exposure to runoff plus an additional 46 h in control water. Runoff toxicity at inflow and outflow of site 1 was high throughout the experiment. At site 2, $\geq$95% amphipod mortality occurred within 4 h of exposure to irrigation runoff during the first few hours of runoff (Table 2), but of those organisms deployed last at inflow and outflow locations (6 h after onset of runoff), $61.0 \pm 10.3\%$ and $15.0 \pm 10.0\%$ (mean $\pm$ SD) survived the 2 h exposure to runoff plus an additional 46 h in control water, respectively. Runoff toxicity at both inflow and outflow of site 2 remained high throughout the experiment and was at times higher at the outflow than at the inflow.

For *H. azteca*, measured in situ TU (based on measured time to 50% mortality) were considerably higher than predicted TU (based on measured insecticide concentrations and LC50), especially for runoff from site 2 containing permethrin (Table 3). Although measured TUs for chlorpyrifos-containing runoff at site 1 were on average 1.8-fold higher than predicted TUs, the difference ranged from 4.2 to more than 26.6-fold for permethrin-containing runoff at site 2.

In situ tests with *H. azteca* showed a modest reduction in runoff toxicity after passage through the ditch at most experimental time points; 13 to 36% (deployment groups 2–5) at site 1, and 3 to 27% (deployment groups 1–3) at site 2. Toxicity was higher at the outflow than at the inflow in the beginning phase at site 1, and in the last phase at site 2. Passage through the

Table 3. Time to 50% *Hyalella azteca* mortality (ET50), measured and predicted toxic units (TU) during in situ exposure to irrigation runoff containing chlorpyrifos or permethrin

Site	Organism deployment group	Measured concentration [μg/L]		ET50 [h]			Measured TU in situ			Predicted TU[a]	
		Inflow	Outflow	Control	Inflow[b]	Outflow[b]	Control	Inflow	Outflow	Inflow	Outflow
1- Alfalfa	1	4.5[c]	2.8[c]	>48	5.3	3.1	<2.0	18.1	31.0	24.2	15.1
	2	2.9[c]	3.1[c]	>48	3.0	4.0	<2.0	32.0	24.0	15.6	16.7
	3	3.3[c]	2.7[c]	>48	2.6	3.0	<2.0	36.9	32.0	17.7	14.5
	4	3.7[c]	2.6[c]	>48	1.8	2.8	<2.0	53.3	34.3	19.9	14.0
	5	4.0[c]	2.7[c]	>48	>2.0[d] (4.3)	>2.0[d] (5.6)	<2.0	<48.0[d] (22.3)	<48.0[d] (17.1)	21.5	14.5
2 –Tomato	1	0.618[e]	0.148[e]	>48	2.9	3.0	<2.0	33.1	32.0	7.9	1.9
	2	0.350[e]	0.200[e]	>48	2.3	2.8	<2.0	41.7	34.3	4.5	2.6
	3	0.324[e]	0.189[e]	>48	1.1	1.5	<2.0	87.3	64.0	4.2	2.4
	4	0.230[e]	0.139[e]	>48	>2.0[d] (>48.0)	<2.0[d] (0.9)	<2.0	<48.0[d] (<2.0)	>48.0[d] (106.7)	2.9	1.8

[a] Based on 96-h median lethal concentrations (LC50) for *H. azteca* of 0.186 μg/L (chlorpyrifos) and 0.078 μg/L (permethrin).
[b] Significantly different from controls in all groups ($p < 0.05$).
[c] Chlorpyrifos.
[d] Organisms were exposed to irrigation runoff for only 2 h; numbers in parentheses were calculated using 24- and 48-h survival after transfer to control water.
[e] Permethrin.

Table 4. Results of 96-h laboratory tests with *Ceriodaphnia dubia* exposed to irrigation runoff

Site	Sampling location	Sampling time	Percent *C. dubia* mortality at sample concentration: 0%	1%	5%	10%	25%	50%	100 %
		July 19, 2007							
1- Alfalfa	Inflow	3:35	0	0	100[a]	100[a]	100[a]	100[a]	100[a]
	Outflow	6:00	0	0	100[a]	100[a]	100[a]	100[a]	100[a]
	Inflow	10:00	0	0	100[a]	100[a]	100[a]	100[a]	100[a]
	Outflow	10:00	0	0	100[a]	100[a]	100[a]	100[a]	100[a]
	Inflow	16:00	0	5	100[a]	100[a]	100[a]	100[a]	100[a]
	Outflow	16:00	0	5	100[a]	100[a]	100[a]	100[a]	100[a]
		August 16, 2007							
2- Tomato	Inflow	11:05	0	0	0	0	10	35	100[a]
	Outflow	13:20	0	5	0	5	0	0	15
	Inflow	16:00	0	0	0	5	5	0	45[a]
	Outflow	16:00	0	0	0	0	0	0	0
	Inflow	18:05	0	0	0	5	5	95[a]	100[a]
	Outflow	18:25	0	5	0	5	0	0	0

[a] Survival was significantly different from control ($p < 0.05$).

vegetated ditch reduced measured insecticide concentrations by an average of 23% (chlorpyrifos, site 1) or 50% (permethrin, site 2; Table 3). This reduction was reflected in predicted TUs, which were significantly lower at the outflow than the inflow locations at both sites (site 1: $p = 0.015$; site 2: $p = 0.047$).

Laboratory toxicity tests—water

No significant reduction in 96-h survival of larval *P. promelas* compared with controls was seen after laboratory exposures to irrigation runoff (data not shown). In contrast, irrigation runoff at site 1 was highly toxic to *C. dubia*. No difference in toxicity was found between samples collected at the inflow and outflow of the experimental ditch (Table 4). Measured TU at site 1 (33.3–34.6 TU) were lower than predicted TU (33–50 TU; Table 5). Irrigation runoff from site 2 was significantly less toxic than that at site 1. In samples from the inflow location, acute toxicity to *C. dubia* was low initially (1.6 TU) and during peak flow ($\sim$ 1 TU), but increased to 2.7 TU at the end of the runoff event. No toxicity to *C. dubia* was detected in samples collected at the outflow. Measured TU at site 2 (<1–2.5) generally agreed with predicted TU for this species (Table 5).

Laboratory toxicity tests—sediments

Sediment collected before the occurrence of runoff at the outflow of the ditch at site 1 was not toxic to *H. azteca* (Table 6). Sediment collected immediately after irrigation runoff significantly reduced amphipod growth, whereas sediment collected 14 d after the runoff event significantly reduced amphipod survival. Measured chlorpyrifos concentrations did not explain the observed toxicity. The concentration present in the nontoxic pre-irrigation sample (65.6 ng/g dry wt) was approximately twice the concentration measured in the toxic postirrigation (day 0; 23.6 ng/g dry wt) sample. All sediment samples collected from the outflow location at site 2 were highly toxic to *H. azteca* (Table 6). Ten-day survival was $1 \pm 1.3\%$ in sediment collected before irrigation, and 0% and $4 \pm 2.6\%$, 2 and 8 d after the runoff event, respectively. Permethrin concentrations in sediments collected before and immediately after the experiment were 7.95 and 12.30 ng/g dry weight.

Control experiments

Temperature tolerance of H. azteca. Test results demonstrate that continuous exposure to 31.0 to 31.7°C for 10 h (the longest duration of field exposures), or exposure to fluctuating temperature extremes (hourly, between 17°C and 33°C, or vice versa) for 10 h had no effect on amphipod survival. Average survival was 95 to 100% in all treatments tested.

Pesticide adsorption to flow-through exposure system. Supplemental Data, Figure S1, shows the results of our experiment measuring insecticide adsorption and loss to the experimental exposure system. Overall, recovery of pesticides from the water was slightly decreased after passage through the system, most notably for cyfluthrin (93–109%), chlorpyrifos (93–107%), cypermethrin (99–107%), deltamethrin (91–109%), and tetramethrin (96–109%). Overall there appeared

Table 5. Measured and predicted toxic units (TU) for *Ceriodaphnia dubia,* exposed in the laboratory to irrigation runoff containing chlorpyrifos or permethrin

Site	Sampling Period	Measured concn. [μg/L] Inflow	Measured concn. [μg/L] Outflow	Measured TU Inflow	Measured TU Outflow	Predicted TU[a] Inflow	Predicted TU[a] Outflow
	July 19, 2007						
1- Alfalfa	Initial	3.4[b]	2.8[b]	33.3	33.3	42	35
	Peak	3.3[b]	2.7[b]	33.3	33.3	41	33
	Final	4.0[b]	2.7[b]	34.5	34.5	50	34
	August 16, 2007						
2- Tomato	Initial	0.618[c]	0.148[c]	1.6	<1.0	2.5	<1.0
	Peak	0.324[c]	0.189[c]	~1.0	<1.0	1.3	<1.0
	Final	0.230[c]	0.139[c]	2.7	<1.0	~1.0	<1.0

[a] Based on 96-h median lethal concentrations (LC50) for *C. dubia* of 0.08 μg/L (chlorpyrifos) or 0.24 μg/L (permethrin).
[b] Chlorpyrifos.
[c] Permethrin.

Table 6. Results of 10-d *Hyalella azteca* toxicity tests on sediments collected at the ditch outflow locations[a]

Site	Sediment sample	Sampling date	*H. azteca* survival [%]		Final dry weight [mg/surviving individual]		Measured insecticide concn. [μg/kg dry wt]
			Mean	SE	Mean	SE	
1- Alfalfa	Pre-irrigation	7/17/2007	94	2.6	0.088	0.006	65.6[b]
	Post-irrigation: day 0	7/19/2007	74	6.0	0.076[c]	0.004	23.6[b]
	Post-irrigation: day 14	8/2/2007	29[c]	7.9	0.162	0.019	-
2- Tomato	Pre-irrigation	8/16/2007	1[c]	1.3	0.110	NA	7.95[d,*]
	Post-irrigation: day 1	8/17/2007	0[c]	0.0	NA	NA	12.30[d]
	Post-irrigation: day 7	8/24/2007	4[c]	2.6	0.185	0.065	-

[a] SE = standard error of the mean ($n = 8$).
[b] Chlorpyrifos.
[c] Significantly different from control ($p < 0.05$).
[d] Permethrin.
[*] Below reporting limit.

to be little loss (<10%), and differences were likely within experimental error margins.

DISCUSSION

The results of this study demonstrate that irrigation runoff from agricultural fields treated with insecticides containing chlorpyrifos or permethrin as active ingredients was highly toxic to aquatic invertebrates but did not cause acute toxicity to larval fathead minnows. Passage through a 389- to 402-m section of vegetated drainage ditch was beneficial and reduced runoff toxicity, but the extent of this remedial effect was relatively small. Runoff containing chlorpyrifos remained highly toxic to both invertebrate test species, and runoff containing permethrin remained highly toxic to *H. azteca* after passage through the ditch (Tables 3 and 5).

The remedial effect of the vegetated ditch on runoff toxicity was smaller than measured insecticide concentrations would suggest, likely, in part, because of additive or synergistic effects of chemicals other than chlorpyrifos or permethrin [24,25]. In situ tests with *H. azteca* showed a modest reduction in toxicity on the order of 15% at both experimental sites, whereas chlorpyrifos (site 1) and permethrin (site 2) concentrations were on average 23 and 50% lower, respectively, at the ditch outflow (Table 3). Similarly, measured *C. dubia* toxicity in runoff containing chlorpyrifos did not indicate a remedial effect of the vegetated ditch (Table 5). Results of sediment toxicity tests with *H. azteca* also suggest that additional toxic chemicals were present at test sites. Permethrin sediment concentrations (7.95–12.3 ng/g dry wt) measured at site 2 were too low to explain the almost 100% amphipod mortality observed. For this species, 10-d LC50s of permethrin range from 127 to 249 ng/g dry weight [26].

The presence of additional chemicals released from the irrigated fields or from ditch sediments, or applied as inert ingredients of pesticide formulations, may, in part, explain the observed discrepancies between measured and predicted TU. Given that more than 900 different pesticides are applied for agricultural pest control in California (California Department of Pesticide Regulation, Sacramento, CA, USA, personal communication) in the form of commercial formulations, likely organisms were exposed to complex mixtures of chemicals. These were either deposited during previous pest control treatments and resuspended during irrigation and runoff or applied as part of the pesticide formulations used before our experiments. Both chlorpyrifos and permethrin have been shown to act additively or synergistically with other environmental contaminants [27–31]. Some formulated pesticide products are known to be more toxic than the pure active ingredients [29,32]. Additional chemical analyses of water samples were performed during our experiment at site 2, because toxicity appeared to increase toward the end of the experiment. Two organochlorine insecticides, dieldrin and DDE (a breakdown product of dichlorodiphenyltrichloroethane), were detected at concentrations of 5 to 8 ng/L dieldrin and 14 to 33 ng/L DDE (inflow), and 8 ng/L dieldrin and 12 to 20 ng/L DDE (outflow). Sediment samples from the same site were collected and analyzed 2 months before our experiments, because this ditch drained irrigation runoff from several fields. Multiple insecticides were detected: chlorpyrifos (3.83 ng/g), dieldrin (1.3 ng/g), DDT (7.64 ng/g), DDE (9.62 ng/g), DDD (1.19 ng/g), and fipronil-sulfone (6.65 ng/g) (Supplemental Data, Table S3). The detection of DDT and its metabolites was unexpected, because this pesticide has been banned in the United States since 1972. Detected concentrations of individual insecticides are, however, far below reported 96-h or 10-d LC50 values for our test species [33–36], and no effect on *H. azteca* survival or growth was observed in 28-d exposures to sediments containing more than 70 ng/g fipronil sulfone [37]. Little is known about the species-specific effects of complex chemical mixtures, and the discussion of potential effects on our test organisms therefore remains speculative.

Aside from potential mixture effects, multiple stressors may have increased toxicity to organisms exposed in situ. Measured in situ TUs for *H. azteca* were considerably higher than predicted TU. Predicted TU for chlorpyrifos-containing runoff were approximately half of the measured TU, but the difference was much greater for runoff from the permethrin-treated field (Table 3). Although the pH showed little variation (7.4–8.4) across sites and exposure systems, high water temperatures later in the day and relatively low dissolved oxygen concentrations (Supplemental Data, Table S1) may have increased toxicity of chlorpyrifos at site 1 [38]. At site 2, oxygen concentrations were well within *H. azteca* tolerance limits at all times, but water temperatures were higher than at site 1; however, the toxicity of pyrethroid insecticides is known to decrease with increasing temperature [39]. Therefore, apart from potential mixture effects discussed previously, measured permethrin concentrations (used to calculate predicted TU) may not have reflected exposure concentrations in the field. This is supported by the relatively good agreement between predicted and measured TU in laboratory tests with *C. dubia* (Table 5). For pyrethroid insecticides, loss is a common concern, because these relatively

hydrophobic chemicals tend to adsorb to sampling equipment and degrade during sample storage [40]. Our data on pyrethroid adsorption to the in situ systems showed little loss of chemical, probably because water was continuously moving through the system; adsorption of pyrethroids to surfaces can be reversed by agitation [40]. However, studies at UCD-ATL have shown that significant amounts of pyrethroids may be lost during routine processing and testing of water samples. For example, only 38% of the initial concentration of permethrin spiked into laboratory control water was detected after a mock sampling procedure and 14-d storage at 4°C in the dark (I. Werner, UCD-ATL, unpublished data). In the current study, laboratory tests with *C. dubia* and fathead minnows were initiated within 24 h of sample collection, and sample storage time for chemical analyses was 4 to 6 d (water) or 16 to 40 d (frozen sediment). Nevertheless, our results raise concerns about the common reliance of environmental risk assessments on laboratory tests and analytical chemistry data, especially with regard to pyrethroid insecticides.

A major finding of this study is that acute laboratory *C. dubia* tests radically underestimated pyrethroid-associated toxicity to *H. azteca* at site 2. Whereas 32 to more than 48 TU were measured for *H. azteca* exposed in situ, only less than 1 to 2.7 TU were measured in acute *C. dubia* laboratory tests. Contrary to that, *C. dubia* was somewhat more sensitive to chlorpyrifos, with 33.3 to 34.5 measured TU for *C. dubia* and 17.1 to 53.3 measured in situ TU for *H. azteca*. *Ceriodaphnia dubia* is approximately 2.5 times more sensitive to chlorpyrifos than *H. azteca,* whereas *H. azteca* is approximately 3 times more sensitive to permethrin (LC50 values provided in Tables 3 and 5), but this does not explain the large difference in toxicity observed in the current study. Loss of chemical during sampling, storage, and testing because of adsorption and degradation is probably more important (see previous discussion). Similar to our findings, a study on runoff from fruit orchards detected toxicity to amphipods exposed in situ, whereas no acute toxicity was observed in standard toxicity tests with *Daphnia pulex* [41]. The *C. dubia* test is widely used for toxicity monitoring programs and considered to be a highly sensitive monitoring tool [21]. Results of the current study show that its exclusive use in toxicity monitoring could yield a significant number of false negatives in environments that receive urban or agricultural runoff, especially where pyrethroid insecticides have become the dominant group of pesticides applied for insect pest control. Water quality regulations based on tests with this species therefore may not be protective of other aquatic invertebrates such as *H. azteca*.

CONCLUSIONS

We have shown that irrigation runoff from two agricultural fields treated with the OP chlorpyrifos or the pyrethroid permethrin was not acutely toxic to larval fathead minnows. However, runoff containing chlorpyrifos was highly toxic to two invertebrate species, the waterflea *C. dubia* and the amphipod *H. azteca*. Runoff containing permethrin was highly toxic to *H. azteca* exposed in situ but nontoxic to moderately toxic to *C. dubia* exposed in the laboratory. Passage through approximately 400 m vegetated drainage ditch reduced toxicity by approximately 15%. Experimental ditch lengths were thus unable to eliminate the risk of irrigation runoff to aquatic ecosystems. However, runoff must travel through additional ditches (field and roadside ditches) before actually entering receiving waterways. Toxicity to *H. azteca* measured in situ was consistently higher than predicted toxicity, in particular for runoff containing permethrin. The combined effects of complex chemical mixtures and, in part, multiple stressors may have caused this discrepancy, and loss of permethrin during sampling and storage may have resulted in low insecticide detection. Laboratory *C. dubia* tests radically underestimated pyrethroid-associated toxicity to *H. azteca* exposed in situ. We conclude that protective measures based on chemical concentrations or laboratory toxicity test with *C. dubia* alone do not ensure adequate protection of aquatic ecosystems from pyrethroid-associated toxicity, are not reflective of field conditions, and do not take into account the deleterious effects of multiple chemicals or stressors.

SUPPLEMENTAL DATA

Figure S1. Percent recovery of pesticides in laboratory water after flowing through the in situ exposure system at 200 L/h (44 KB PDF).

Table S1. Mean plant densities (±standard deviation [SD]) and percent cover (±SD) in experimental drainage ditches.

Table S2. Water quality parameters measured in in situ exposure systems; mean ± standard error (maximum-minimum).

Table S3. Results of chemical analysis of pre-irrigation sediment samples collected from the experimental drainage ditch at site 2 on May 4, 2007 (17 KB PDF).

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